BATCH EXTRACTION OF CAFFEINE FROM COCOA MCBC 1

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ABSTRACT

Caffeine is naturally occurring substances found in the leaves seeds of fruits of a group of compounds known as methylxanthines. The most commonly known source of caffeine is coffee and cocoa seeds. However, cocoa seeds have received limited attention as a source for caffeine since it widely used as a source to make a cocoa powder and chocolate. The caffeine extraction from cocoa seeds will be another way to fulfill demand for caffeine. The objectives of this research is to extract the caffeine from Cocoa MCBC I Seeds by the effect of different extraction time, particle size of cocoa seeds powder and ratio of cocoa seeds-solvent towards yields of caffeine by using batch system. This research was divided into five main steps which are the sample preparation, leaching of caffeine, liquid-liquid extraction, drying of caffeine and analysis the crude caffeine using High Performance Liquid Chromatography (HPLC). Extraction yield increase towards the increasing of extraction time. Meanwhile, extraction yield of caffeine increased when using the smallest particle size of cocoa. However, the best ratio of cocoa seeds-solvent to get the highest extraction yield was 1:5. Besides that, either using lower ratio or higher ratio than 1:5, the extraction yield cannot achieve as higher as 1:5 cocoa-solvent ratio. As a conclusion, caffeine can be extracted from cocoa MCBC 1 seeds by using batch system. 60 minutes of extraction time, 400 of the particle size of cocoa seeds powder and 1:5 ratio of cocoa seed-solvent gives highest extraction yield of caffeine.

ABSTRAK

Kafein adalah zat alami yang ditemui dalam daun, biji buah-buahan dari kumpulan sebatian yang dikenali sebagai methylxanthines. Sumber yang biasa bagi kafein adalah kopi dan biji koko. Namun begitu, biji koko kurang mendapat perhatian sebagai sumber kafein kerana banyak digunakan sebagai sumber untuk membuat serbuk koko dan coklat. Ekstrak kafein daripada koko merupakan satu cara lain untuk memenuhi permintaan terhadap kafein. Penyelidikan ini bertujuan untuk mengekstrak kafein daripada biji koko MCBC1 dengan memanipulasi waktu ekstrak, saiz serbuk koko dan nisbah biji koko-pelarut terhadap hasil kafein dengan menggunakan sistem batch. Penyelidikan ini dibahagikan kepada lima bahagian utama iaitu penyediaan sampel, ekstrak kafein dari sampel ke cecair, ekstrak ceceir ke cecair, pengeringan kafein dan analisi kafein menggunakan *High Performance Liquid Chromatography (HPLC)*. Hasil ekstrak meningkat seiring penigkatan masa ekstrak. Namun begitu, hasil ekstrak menurun apabila saiz sampel meningkat. Nisbah biji koko-pelarut yang terbaik yang mendapatkan hasil ekstrak yang tinggi adalah 1:5. Kesimpulannya, kafein boleh dihasilkan dari biji koko MCBC 1 melalui sistem batch. 60 minit masa ekstraksi, 400 dari saiz zarah serbuk biji koko dan nisbah 1:5 biji koko-pelarut memberikan hasil ekstraksi tertinggi kafein.

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LIST OF ABBREVIATIONS/TERMINOLOGY/SYMBOLS

º	- Degree
%	- Percent
µg/ml	- Microgram per milliliter
µm	- Micrometer
C	- Celsius (Temperature)
et al.	- And others
g	- Gram
GC	- Gas Chromatography
HPLC	- High Performance Liquid Chromatography
m	- Meter
MCBC	- Malaysian Cocoa Board Clone
min	- Minute
ml	- Milliliter
mg	- Milligram
M	- Mole
SPE-HPLC	- Solid Phase Extraction- High Performance Liquid Chromatography

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CHAPTER 1

INTRODUCTION

This introduction gives the idea about the rationale and general understanding of the research. This chapter discovers the subtopic of background of study problem statement research objectives, scope of research and significance of research.

1.1 Background of Study

The quality of the cocoa beans depends on many factors such as the genotype, the agronomic management, the soil factors, the climatic conditions, and the most importantly the post harvest technology (Brunetto et al., 2007). Cocoa based foods are consumed worldwide and have been shown to be very nutritious, containing substantial amounts of amino acids, except methionine and arginine. Vitamins, minerals and fat are also presence a high proportion. Cocoa products contain many physiologically active compounds. The high level of fat contributes to the high gross energy content of the cocoa bean. Despite its high with nutrition value, the presence of caffeine and thebromine alkaloids may limits its potential as a nourishing food (Beckett, 1988).

Caffeine is naturally occurring substance found in the leaves, seeds or fruits of over 63 plants species worldwide and is part of a group of compounds known as methylxanthines. The most commonly known sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves. Caffeine is pharmacologically active substance and depending on the dose, can be mild central nervous system stimulant. Caffeine does not accumulate in the body over the course of time and is normally excreted within several hours of consumption (Barone and Roberts, 1996).

Decaffeination is a popular term in present modern world in order to optimize the caffeine contains in various sources. This is simply use of a solvent, which extract caffeine. For this purpose, the currently available solvents are chloroform, methyl chloride, ethyl acetate, super critical carbon dioxide and others (Mumin et al., 2006).

1.2 Problem Statement

Caffeinated products have been consumed by humans since prehistoric times. Today, most of the world's population, regardless of geographical location, gender, age, or culture consumes caffeine daily. With increasing use of caffeine in the beverage and pharmaceutical industries, demand for caffeine continues to increase. Natural sources of caffeine used today include tea leaves and coffee beans. A naturally occurring source of caffeine that has received limited attention is the seed of cocoa tree. This is because nowadays, cocoa are widely used to make cocoa powder and chocolate. The caffeine extraction from cocoa seeds will be another way to fulfill demand for caffeine.

1.3 Objectives

The current research is done to achieve the following objectives:

i) To extract the caffeine from cocoa MCBC I seeds using batch system.

ii) To investigate the effect of extraction time, particle size of sample and cocoa seed-solvent ratio towards extraction yield of caffeine.

1.4 Research Scope

The scopes of this research are:

i) Extraction of caffeine using batch system.

ii) Investigate effect of extraction time, particle size of sample and cocoa seed-solvent ratio towards extraction yield of caffeine.

iii) Analysis of crude caffeine using High Performance Liquid Chromatography (HPLC).

1.5 Significance of Research

This research created another application for cocoa seeds except as a chocolate and cocoa powder. Cocoa also can be use as a raw material for caffeine. It can fulfill demand for caffeine. Extract the caffeine composition in this research is from new breed which is Cocoa *MCBC I*.

CHAPTER 2

LITERATURE REVIEW

2.1 Cocoa

The cocoa tree also known as Theobroma Cocoa is a native of the dense tropical forests of the Amazon where it grows in conditions of semishade, warmth, and high humidity. The genus of Theobroma consists of over twenty species.

Botanically, the term "cocoa" refers to the tree and its fruits. Cocoa describes the bulk commercial dried fermented beans, as well as the powder produced from the beans. The tree can only be cultivated within fairly narrow limits of altitude, latitude and humidity.

The cultivation of 75 percent of the world's cocoa lies within eight degree of either side of the equator, with exceptions in some areas to about 18 degrees north or south. The optimum growing temperature for cocoa tree is between 18 to 32ºC. (Wood, 1985).

Table 2.1: Chemical Composition of Cocoa Beans

	Knapp and Churchman (1937)		Fincke (1965)		Jensen (1931)		Pearson (1981)			
							Nib, %		Shell, %	
	Nib, %	Shell, %	Nib, %	Shell, %	Nib, %	Shell, %	Max	Min	Max	Min
Water*	2.1	3.8	5	11	3.9	8.1	3.2	2.3	6.6	3.7
Fat (cocoa butter, shell fat)	54.7	3.4	54	3	63.2	3	57	48	5.9	1.7
Ash	2.7	8.1	2.6	6.5	3.1	7.6	4.2	2.6	20.7	7.1
Nitrogen										
Total nitrogen	2.2	2.8	2.1	2.6		2.6	2.5	2.2	3.2	1.7
Protein nitrogen	1.3	2.1								
Theobromine	1.4	1.3	1.2	0.8	1.3		1.3	0.8	0.9	0.2
Protein			11.6	13.5	13.9	15.9				
Caffeine	0.07	0.1	0.2				0.7	0.1	0.3	0.04
Carbohydrates										
Glucose	0.1	0.1	1							
Sucrose	0	0								
Starch	6.1	No true starch	6		6		9	6.5	5.2	3.4
Pectine	4.1	8								
Crude fiber	2.1	18.6	2.6	16.5	2.7	14.8	3.2	2.2	19.2	12.8
Cellulose	1.9	13.7	9							
Pentosans	1.2	7.1	1.5	6	1.4	8				
Mucilage and gums	1.8	9								
Tannins				9						

* Water content can vary according to the degree of drying or roasting.

Source: Bernard, 1999

Cocoa tree grow from seed through three stages of development. The early stages of grow involve the initial growth of the seed. The cocoa initially germinates and pushes the cotyledons about an inch above the surface of the soil and then it produces the first leaves.

The next stage gives the plant further vertical growth until fan branches appear. The number of these sideways growths can vary depending on the variety. The growth of the fan branches forms a jorquette. Further vertical growth depends on chupons, the branches that normally develop on the main trunk and that grow up above the fan branches. The chupons may then form a new jorquette with their own fan branches. The height of the tree is typically between eight and ten meters (Dand., 1999).

Figure 2.1: Fermented and Roasted Cocoa

The most visible use of cocoa is in candies and drinks. Its solids form are ground into powder and its oil is extracted and converted into cocoa butter, these two ingredients can be mixed with sugar, milk, flour and a variety of other common goods to create chocolate bars, cakes and other sweets. In addition, raw cocoa beans are sometimes eaten for their flavonoids to improved cardiovascular health (Eric et al., 2006).

Cocoa butter is used for pharmaceuticals. It is used for encapsulating certain drugs because it can be stored sadely and dissolved readily in the body. Naturally resists rancidity compound in cocoa makes it ideal for products such as cosmetics and soap. A study by Young and Jewell shown that topical application of vitamin E in cocoa can be used to reduce stretch marks and scar removal.

Table 2.2: Composition of Cocoa versus Chocolate (in %)

	Cocoa Beans	Cocoa Butter	Dark Chocolate
Proteins	18.0	6.4	6.0
Lipids	56.0	54.0	27.0
Carbohydrates	13.5	28.0	54.0
Water	3.0	2.0	1.0
Theobromine	1.45	1.1	0.5
C1.1affeine	0.05	0.5	0.07
Theophylline	-	-	0.001

*This average values are subject to considerable variations
Source: Hesse, 2002

2.2 Caffeine

Caffeine is the world's most popular drug, easily surpassing nicotine and alcohol. Caffeine is the only addictive psychoactive substance that has overcome resistance and disapproval around the world that it is freely available almost everywhere, sold without license and even added to beverages intended for children (Bennet and Bonnie, 2001).

Caffeine (1,3,7-trimethylxanthine, guaranine) is a plant-derived alkaloid. Caffeine, theophylline, theobromine and paraxanthine are usually easily to detect in toxicological samples due to dietary exposure to caffeine. The average cup of coffee or tea in United State is reported to contain between 40 and 150 mg of caffeine

(Baselt, 2004) although special coffees may contain much higher doses of caffeine (McCusker, 2003).

Caffeine is an alkaloid of the methylxanthine family. In its pure state, it is an intensely bitter white powder. Its chemical formula is $C_8H_{10}N_4O_2$, its systematic name is 1, 3, 7-trimethylxanthine (Arnaud, 1987) and its chemical formula is shown below.

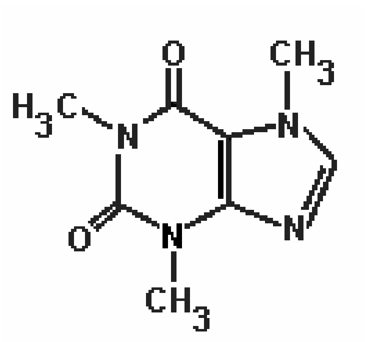

Figure 2.2: Structure of Caffeine

Pure caffeine occurs as odorless, white, fleecy masses, glistening needles of powder. Its molecular weight is 194.19g, melting point is 236 0C, point at which caffeine sublimes is 1780C at atmospheric pressure, pH is 6.9 (1% solution), specific gravity is 1.2, volatility is 0.5%, vapor pressure is 760 mm Hg at 178 0C, solubility in water is 2.17%, vapor density 6.7 (Clementz and Dailey, 1988).

Methylxanthine or caffeine contents in many food and beverages have been determined, including coffee, tea, carbonated beverages, some chocolate products, caffeinated water and chewing gum (Hoch, 1998). Most of the research has focused on the caffeine content in coffee, tea, and cola beverages.

However, less data exist on the methylxanthine contents in chocolate foods and beverages. Caffeine and theobromine contents have been reported for some chocolate products including commercial hot chocolate, bakery products, chocolate milk, and cocoa powder. However, methylxanthine concentration data in cocoa are presented as an average of several unspecified brands or varieties and do not provide detailed information on specific products (Austin et al., 2001).

Austin et al., 2001 investigated that the methyxanthine contents in the chocolate products (cereal and toaster pastry) were lower than other chocolate dessert products such as pudding and cookies. However, the amount of caffeine in all the product tested was quite low as compared with coffee, tea, and cola products.

Caffeine is among the drugs that have been deliberately used for stimulants effects by athletes. It is listed as a banned drug by the International Olympic Committee because of its effects upon performance. Caffeine can be used wisely or it can be used foolishly like any other drug. People who take excessive amount of caffeine through their consumption of tea and coffee can experience various unpleasant side effects as a result. Usually they fail to recognize these as toxic effects. Caffeine can cause headaches, insomnia, dizziness, trembling, diarrhea, breathlessness and anxiety symptoms. It also has various toxic effects on the heart. These can include palpitations, rapid pulse or irregular pulse rate, and when consumed in large amounts, it can lead to increased blood pressure (Gossop, 2007).

Since it acts as stimulants of the central nervous, muscle and circular system, this compound has become a valuable agent for human body. Although caffeine has been extensively consumed in pharmaceuticals, soft beverages, etc, it is creates health risk in children, pregnant women and some patient. Therefore, obtaining of caffeine and decaffeination of food become highly important. It can be synthesized from chloroacetic acid or uric acid (Metin and Hacer, 2004., 2009).

Table 2.3: Caffeine Content of Food Products as Reported by Various Sources.

Product	Volume or weight*	Caffeine content (mg) Range	Average	Reference
Roasted and				
ground coffee	150 ml	64–124	83	Burg (1975)
(percolated)†	150 ml	—	74	Gilbert *et al.* (1976)
	‡	37–128	82	Stravric *et al.* (1988)
Instant coffee	150 ml	40–108	59	Burg (1975)
	150 ml	—	66	Gilbert *et al.* (1976)
	150 ml	—	66	US FDA (1980)
	‡	21–117	71	Stavric *et al.* (1988)
Roasted and				
ground coffee	150 ml	2–5	3	Burg (1975)
(decaffeinated)	150 ml	—	2	Gilbert *et al.* (1976)
Instant coffee	150 ml	2–8	3	Burg (1975)
(decaffeinated)	‡	2–6	4	Stavric *et al.* (1988)
Roasted and				
ground coffee	150 ml	—	112	Gilbert *et al.* (1976)
(drip)	‡	37–148	84	Stavric *et al.* (1988)
All coffee				
(except				
decaffeinated)	150 ml	29–176	—	Gilbert *et al.* (1976)
Tea	150 ml	8–91	27	Gilbert *et al.* (1976)
	150 ml	—	24	Stavric *et al.* (1988)
Bagged tea	150 ml	—		Burg (1975)
	150 ml	28–44	—	US FDA (1980)
	150 ml	—	30	Wheeler (Thomas J. Lipton Inc., 1989, personal communication)
*Leaf tea	150 ml	30–48	41	Burg (1975)
	150 ml	—	30	Wheeler (Thomas J. Lipton Inc., 1989, personal communication)
Instant tea	150 ml	24–31	28	Burg (1975)
	150 ml	—	20	Wheeler (Thomas J. Lipton Inc., 1989, personal communication)
Cocoa—African	150 ml	—	6	Burg (1975)
—South American	150 ml	—	42	Burg (1975)
Cocoa	150 ml	—	5	US FDA (1980)
	150 ml	2–7	4	Zoumas *et al.* (1980)
Chocolate bar	28 g	—	20	Gilbert *et al.* (1976)
Milk chocolate	28 g	—	6	US FDA (1980)
	28 g	1–15	6	Zoumas *et al.* (1980)
Sweet chocolate	28 g	5–35	20	Zoumas *et al.* (1980)
Chocolate milk	240 ml	2–7	5	Zoumas *et al.* (1980)
Baking chocolate	28 g	—	35	US FDA (1980)
	28 g	18–118	60	Zoumas *et al.* (1980)
Chocolate candy	28 g	1.5–6	—	Tarka (Hershey Foods, 1989, personal communication)
Soft drinks				
Regular colas	180 ml	15–24	—	NSDA (1993, unpublished data)
Caffeine-free colas	180 ml	0	—	NSDA (1993, unpublished data)
Diet colas	180 ml	13–29	—	NSDA (1993, unpublished data)
Caffeine-free				
diet colas	180 ml	0	—	NSDA (1993, unpublished data)
Speciality products	180 ml	0–35	—	NSDA (1993, unpublished data)
Others	180 ml	0	—	NSDA (1993, unpublished data)

*All volumes and weights reported by original sources have been converted to metric units for consistency.
†The US FDA cites a range of 75 to 155 mg caffeine per cup (150 ml or 5 fl. oz) of coffee, noting that percolated coffee is in the lower and drip coffee in the upper part of this range.
‡The volumes of coffee per serving prepared at home in this study ranged from 25 ml to 330 ml with a mean of 224 ml (about 7.5 fl. oz). Volumes of commercially prepared coffee ranged from 30 ml to 205 ml with a mean of 171 ml.

Sources: Barone and Roberts, 1996

2.3 Decaffeination

The extraction of caffeine from coffee beans is one of the first Chemical Engineering process. The patent of this process exist since 1905 (Bichel, 1979). The extraction of caffeine from coffee is a solid-liquid process, where the coffee is a transferred from the coffee beans solid matrix to the bulk solvent. The beans are not spherical and the caffeine concentration is time depending during process in both phases (Espinoza-Perez et. al., 2007).

Several investigations were report for extraction by supercritical carbon dioxide from guarana (Mehr et al., 1996; Saldana et al., 2002), coffee beans (Peker et al., 1992), green tea (Chang et al., 2000, Park et al., 2007).

Also, Saldana et al., 2002 investigate that methylxanthines were extracted from guarana seeds, mate leaves and cocoa seeds using supercritical carbon dioxide and ethanol. Furthermore the extraction of tea polyphenols and tea caffeine from green tea leaves were investigates by the microwave-assisted extraction method.

The present experimental study was carried out on the stalk and fiber wastes of Turkish black tea factories by supercritical carbon dioxide extraction. The nature of the raw materials makes leaching of caffeine from tea stalk and fiber wastes economic and commercially feasible. They have no economic value other than being used as very low grade fuel. The purpose of this study was to investigate the influence of operational conditions on extraction yield. The yield was compared with the yield of chloroform extraction in the specified conditions which had been reported in a previous study (Metin and Hacer, 2004).

During the testing in Shufen et. al., 1990, it was found that only 1 ml of 1 M sodium hydroxide solution was needed to regulate the pH between 12.5 - 12.7 for pure sample solution. However, when it was done according to the procedure, over five times of 1 M sodium hydroxide solution used to give the same pH. The reason may be that the presence of sodium hydrogen carbonate and sodium hydroxide creates a buffereing effect, making control of the pH easier.

The extraction of methylxanthines has been performed using liquid extraction solvents such as dimethyl chloride, chloroform and water (Hulbert et al., 1998, Caudle et. al., 2001). However, chemical solvent need several time for extraction complete. Although water an excellent solvent, is highly non selective and its use may result in the removal of other valuable component from the extracted product, which gradually leads to deterioration of the analytical column (Saldana et al., 2002).

Kim et. al., 2008 said that acetone, methanol, ethanol and acetonitrile as extraction solvents were used to obtain caffeine-free green tea. Also, mixture of methanol and water was used to extract catechin from green tea. The use of the organic solvents in not appropriate because residual organic solvent have potential adverse effect on human health even though effective decaffeination can be achieved using the organic solvents. Therefore, non-toxic and effective decaffeination alternatives such as supercritical fluid extraction using carbon dioxide as a solvent have been explored in recent years. The advantage on using supercritical carbon dioxide extraction ($SCCO_2$) have been well documented by Mansoori et al., 1988, Park et al., 1987, and Martinelli et al., 1991.

2.4 Analysis of Caffeine

HPLC has recently used to identify and quantify simultaneously methylxanthine and polyphenols levels in cocoa and chocolate products (Blauch and Tarka, 1983; Kim et al., 1983; Kreiser and Martin, 1980). Most of the research carried out involved the analysis of commercial cocoa, chocolate liquors, different type of chocolate and cocoa beverages (Timbi et al., 1978).

Pura, 2001 proposed the use of Seppak cartridge for the purification of cocoa extract before injection onto a HPLC reverse-phase column. In this way, the interfering cocoa pigments are effectively removed, therefore increasing the column life.

Mumin et al., 2006 have developed a HPLC method for the determination of caffeine which was using HPLC instead of using UV-Visible Spectrophotometer. HPLC method is choosing to determine the caffeine because HPLC is the most widely used qualitative and quantitative determination and separation method.

The method is popular because it is non-destructive and can be applied to thermally labile compounds (unlike GC). It is also a very sensitive technique since it incorporates a wide choice of detection methods.

With the use of post-column derivation methods to improve selectivity and detection limits, HPLC can easily be extended to trace determination of compounds that do not usually provide adequate detector response. The wide applicability of HPLC as a separation method makes it a valuable separation tool in many scientific fields.

Espinoza-Perez et. al., 2007 investigate that the evolution of caffeine concentration was measured both in extract and refined phase by using gas chromatography.

CHAPTER 3

METHOD AND METHODOLOGY

This method of extraction of caffeine was using batch system. This method of this study is based on the study of which the title is Determination and Characterization of Caffeine in Tea, Coffee and Soft Drinks by Solid Phase Extraction and High Performance Liquid Chromatography (SPE - HPLC) (Mumin et al., 2006) and UV Spectrophotometric Determination of Theobromine and Caffeine in Cocoa Beans (Li et al, 1990).

3.1 Material

The green *MCBC1* cocoa seeds were bought from Malaysian Cocoa Board research station in Jengka, Pahang.

3.2 Chemicals

There are list of the chemicals was used in order to done this research:

- Ethanol
- Solid sodium hydrogen carbonate
- 1M sodium hydroxide
- 10% lead ethanoate solution

- Solid anhydrous sodium sulfate
- Distilled water

3.3 Apparatus

There are list of the apparatus was used in order to done this research:

- Beaker (50ml, 100ml, 500ml)
- Separatory funnel (500ml).
- Thermometer
- Measuring cylinder (50ml, 250ml)
- Retort stand
- Stopwatch
- Filter paper
- Syringe
- Conical flask 500ml
- Tray
- Spatula
- Weighing boat
- Volumetric flask (10ml, 100ml)

3.4 Equipments

There are list of the equipments was used in order to done this research:

- Hot plate
- Oven
- Siever
- pH meter
- Water bath